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Inhibition of Protein Synthesis *in vitro* by Proteins from the Seeds of *Momordica charantia* (Bitter Pear Melon)

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1. A haemagglutinating lectin was purified from the seeds of *Momordica charantia* by affinity chromatography on Sepharose 4B and on acid-treated Sepharose 6B. It has mol.wt. 115000 and consists of four subunits, of mol.wts. 30500, 29000, 28500 and 27000. 2. The lectin inhibits protein synthesis by a rabbit reticulocyte lysate with an ID_{50} (concentration giving 50% inhibition) of approx. 5μg/ml. Protein synthesis by Yoshida ascites cells is partially inhibited by the lectin at a concentration of 100μg/ml. 3. From the same seeds another protein was purified which has mol.wt. 23000 and is a very potent inhibitor of protein synthesis in the lysate system, with an ID_{50} of 1.8ng/ml. This inhibitor has no effect on protein synthesis by Yoshida cells, and has no haemagglutinating properties. 4. *Artemia salina* ribosomes preincubated with the lectin or with the inhibitor lose their capacity to perform protein synthesis. The proteins seem to act catalytically, since they inactivate a molar excess of ribosomes. 5. The lectin and the inhibitor are somewhat toxic to mice, the LD_{50} being 316 and 340μg/100g body wt. respectively.

Several proteins of plant origin are powerful inhibitors of protein synthesis, and can be divided into two categories. The first includes three highly toxic lectins, ricin, abrin (review by Olsnes & Pihl, 1977) and modeccin (Refsnes *et al.*, 1977; Stirpe *et al.*, 1978), which inhibit protein synthesis in intact cells and in cell-free systems. The second category includes several proteins scarcely toxic to animals, which inhibit protein synthesis in cell-free systems, but have little or no effect on whole cells. These are a '*Phytolacca americana* peptide' (Obrig *et al.*, 1973), crotins and curcins (Stirpe *et al.*, 1976), a protein from wheat germ (Stewart *et al.*, 1977), and a number of unidentified proteins from seeds (Gasperi-Campani *et al.*, 1977, 1980). It is well established that the toxic inhibitors act by inactivating irreversibly and in a catalytic manner (i.e. enzymically) the 60S ribosomal subunit, which becomes unable to bind elongation factor 2 (Sperti *et al.*, 1973; Montanaro *et al.*, 1978; Olsnes & Abraham, 1979). The non-toxic proteins when purified (Irvin, 1975) or semipurified (Sperti *et al.*, 1976) act in the same way, except for the inhibitor from wheat germ, whose action is ATP-dependent (Stewart *et al.*, 1977).

Some non-toxic haemagglutinating lectins also have an inhibitory effect on protein synthesis. Saltvedt (1976) reported that the haemagglutinin from *Ricinus communis* seeds (distinct from ricin) inhibits protein synthesis in HeLa cells, although at higher concentrations than ricin. This effect was due to an A' subunit of the lectin, which was a potent inhibitor of protein synthesis in a cell-free system. Lin *et al.* (1978) purified two lectins from the seeds of *Momordica charantia* (bitter pear melon, a cucurbit): one of these, called *Momordica charantia* agglutinin, was a potent haemagglutinin, whereas the other one, called momordin, had less haemagglutinating power and was a moderate inhibitor of protein synthesis by Ehrlich ascites-tumour cells. Barbieri *et al.* (1979) observed a marked inhibition of protein synthesis in a lysate of rabbit reticulocytes with a haemagglutinin from the seeds of *Momordica charantia* and with other haemagglutinating lectins from seeds and from a fish roe.

We report now that the inhibitory activity on protein synthesis of the extracts from *Momordica charantia* seeds (Gasperi-Campani *et al.*, 1980) is due to the haemagglutinating lectin (referred to here as *Momordica charantia* lectin, or the lectin) and to a much more potent protein, referred to here as *Momordica charantia* inhibitor, somewhat similar to momordin (Lin *et al.*, 1978), although devoid of haemagglutinating activity. Some properties and the

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mechanism of action of these two proteins were studied, and it was ascertained that they act on ribosomes in a catalytic manner, the lectin having little effect and the inhibitor no effect on protein synthesis by Yoshida ascites-tumour cells. Both the lectin and the inhibitor are some 100-fold less toxic to animals than ricin and the related toxins, although they have, respectively, a comparable or a greater effect on protein synthesis in cell-free systems.

Experimental

Materials

Momordica charantia seeds, originally from India, were obtained from Mr. G. G. Celo, Zweibrücken, West Germany. L-[^{14}C]Leucine (specific radioactivity 342mCi/mmol) and [*methyl*-^{3}H]-thymidine (specific radioactivity 20Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. L-[^{14}C]Phenylalanyl-tRNA (mixed charged tRNA containing 0.260 μCi of L-[^{14}C]phenylalanine/mg of tRNA; 414mCi/mmol of L-phenylalanine; percentage of L-phenylalanine-acceptor tRNA bound to L-phenylalanine, 36.9) was from New England Nuclear Corp., Boston, MA. U.S.A.

Bovine serum albumin and trehalose were from Sigma Chemical Co., St Louis, MO, U.S.A.; all other sugars were from the same sources as described by Falasca *et al.* (1979). Neuraminidase was from Behringwerke, Marburg/Lahn, West Germany (one unit is defined as the amount of enzyme required to release 1 μg of *N*-acetylneuraminic acid from human α_1-acid glycoprotein in 15min at 37°C); reference proteins for molecular-weight determinations and poly(U) were from Boehringer Mannheim G.m.b.H., Mannheim, West Germany.

Sepharose 4B, Sepharose 6B, Sephadex G-150 and CM-Sephadex C-50 were from Pharmacia Fine Chemicals, Uppsala, Sweden. Acid-treated Sepharose 6B was prepared as described by Ersson *et al.* (1973), with a 3h acid hydrolysis.

All other chemicals were from the same sources as in previous work (Gasperi-Campani *et al.*, 1978; Montanaro *et al.*, 1978).

Toxicity experiments

The toxicity of the purified proteins was evaluated in Swiss mice of both sexes, weighing 28–30g (males) and 20–22g (females). Animals received food and water *ad libitum*. The proteins, dissolved in 0.9% NaCl, were injected intraperitoneally, at five or six different concentrations, into groups of six animals for each dose. Concentrations ranged from 10μg to 1mg/100g body wt., with a ratio between doses of 3.162 for the lectin, and from 100μg to 1.78mg/100g body wt., with a ratio between doses of 1.778 for the inhibitor. The lectin was also given at a single dose (1mg/100g body wt.) to male Wistar rats weighing 100–120g. LD_{50} was evaluated by the method of Spearman-Kärber as described by Finney (1964).

Assay of haemagglutinating and mitogenic activity

Erythrocytes were collected and washed as described by Falasca *et al.* (1979), and were treated with formalin as described by Butler (1963). Haemagglutination tests were performed in Greiner microtitre plates, U-shaped (Microcult M29; Pool Bioanalysis Italiana, Milan, Italy). Each well contained, in a final volume of 100μl: serial dilutions (by doubling, starting from 50μg/well) of the preparations to be tested, 2.5 units of neuraminidase (when present), the sugars assayed for inhibitory activity, and 50μl of a suspension of fresh (1.2%, v/v) or formalin-treated (2%, v/v) erythrocytes. All solutions and suspensions were in 0.14M-NaCl containing 20mM-sodium phosphate buffer, pH7.2, and bovine serum albumin (15μg/ml). Comparable results were obtained with fresh or formalin-treated erythrocytes. Haemagglutination was evaluated visually after at least 1h at room temperature (20°C). A haemagglutinating unit is defined as the lowest concentration, in μg/ml, giving visible agglutination.

Mitogenic activity was assayed from the incorporation of [*methyl*-^{3}H]thymidine into human peripheral-blood lymphocytes in culture as described by Falasca *et al.* (1979).

Protein synthesis

Protein synthesis was determined from the incorporation of [^{14}C]leucine as described by Gasperi-Campani *et al.* (1978), with Yoshida AH-130 ascites-tumour cells, transplanted in Wistar rats and collected 6–8 days after inoculum, or with a lystate of rabbit reticulocytes prepared as described by Allen & Schweet (1962).

Poly(U)-directed polymerization of phenylalanine was assayed with KCl-washed *Artemia salina* ribosomes prepared as described by Sierra *et al.* (1974). The reaction mixtures (Montanaro *et al.*, 1978) contained, in a final volume of 0.25ml: 80mM-Tris/HCl buffer, pH7.4, 120mM-KCl, 7mM-magnesium acetate, 2mM-dithiothreitol, 2mM-GTP, 25pmol of [^{14}C]phenylalanyl-tRNA, 200μg of poly(U), 250μg of 'S-105 supernatant' (Sierra *et al.*, 1974), and ribosomes. Incubation was at 24°C for 30min. The reaction was arrested with 0.25ml of 10% (w/v) trichloroacetic acid, and the samples were treated as described by Montanaro *et al.* (1978) for the determination of hot-acid-insoluble radioactivity.

Electrophoresis and determination of molecular weight and of isoelectric point

Polyacrylamide-gel electrophoresis was performed at pH4.5 (Reisfeld *et al.*, 1962), at pH7.0

(Williams & Reisfeld, 1964) and at pH 9.5 (Liao *et al.*, 1969). Gels were stained with Coomassie Blue and destained as described by Weber & Osborn (1969).

For molecular-weight determinations, discontinuous sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out in slabs (1.5 mm × 10 cm × 14 cm) in the model 220 Vertical Slab Electrophoresis Cell (Bio-Rad Laboratories, Richmond, CA, U.S.A.) with the Laemmli (1970) system as described in the instruction manual. Before being placed in the wells of the stacking gel, samples (10–30 μg of protein) in 50 mM-Tris/HCl buffer, pH 6.8, were heated for 3 min at 90°C in the presence of 1% sodium dodecyl sulphate, 1% glycerol and 0.0025% Bromophenol Blue; 2-mercaptoethanol (5%, v/v) was either absent or present. Electrophoresis was for 6 h at 25 mA per slab. Soyabean trypsin inhibitor (mol.wt. 21500), bovine serum albumin (mol.wt. 68000) and RNA polymerase (α-chain, mol.wt. 39000; β-chain, mol.wt. 155000; β'-chain, mol.wt. 165000) were used as calibration proteins.

Gel isoelectric focusing was carried out essentially as described by Catsimpoolas (1968) in glass tubes (3 mm × 130 mm) filled with 0.75 ml of gel–protein medium (10–50 μg of protein) prepared by mixing 0.3 vol. of a solution containing 25% (w/v) acrylamide and 1% (w/v) *NN'*-methylenebisacrylamide, 0.075 vol. of 7% (w/v) ammonium persulphate, 0.075 vol. of *NNN'N'*-tetramethylethylenediamine diluted 1:50 with water, and 0.9 vol. of protein sample. After polymerization, isoelectric focusing was performed for 30 min at 250 V and overnight at 150 V. Gels were stained with 0.1% Coomassie Blue in 50% (w/v) trichloroacetic acid for 20 min, destained with several changes of 40% (v/v) methanol at 50°C, and were finally stored in 7.5% acetic acid. The pH gradient was measured in each run on unstained gels as described by Wrigley (1968).

Other determinations

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard, or spectrophotometrically (Kalb & Bernlohr, 1977).

Total neutral-sugar content was determined by the anthrone method (Spiro, 1966) with D-galactose as a standard, after extensive dialysis of the proteins against water. The sugar content was determined also after denaturation of the lectin with 6 M-guanidinium chloride, to avoid the possibility of contamination with residual galactose. For this the lectin solution was dialysed against 0.1 M-Tris/HCl buffer, pH 8.5, then made 6 M with guanidinium chloride, was boiled for 3 min in a water bath, kept at 37°C for 2 h, and finally was dialysed against 1000 vol. of water.

Radioactivity was measured with a Packard Tri-Carb liquid-scintillation spectrometer with an external standard, with a counting efficiency of approx. 80% for ^{14}C and 40% for ^{3}H.

Results

Purification of the lectin and of the inhibitor

Seeds were shelled and were extracted six to eight times with diethyl ether in a blender, and the result-

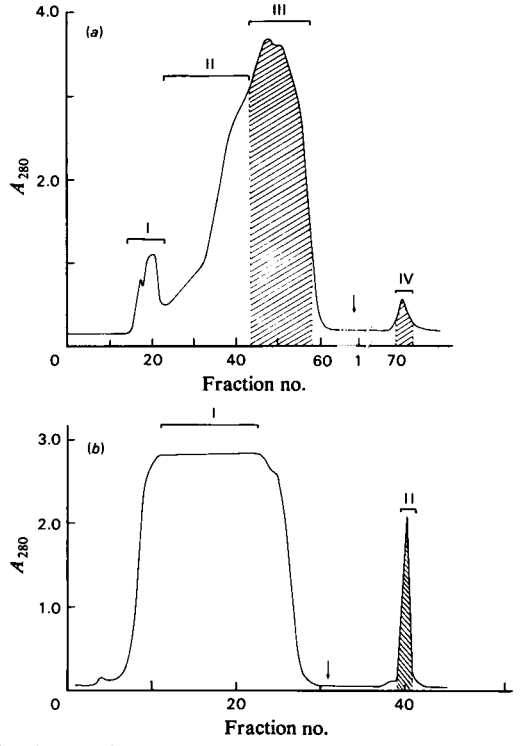

Fig. 1. *Purification of Momordica charantia lectin by affinity chromatography*

(*a*) A portion (85 ml, containing 5.95 g of protein) of the precipitate obtained at 30–60% saturation of $(NH_4)_2SO_4$, redissolved in and dialysed against 0.2 M-NaCl containing 5 mM-sodium phosphate buffer, pH 7.2, was applied to a column (65 cm × 4 cm) of Sepharose 4B, previously equilibrated with the same solution. After washing, the column was eluted with 0.1 M-galactose in the same NaCl/buffer (arrow). Fractions (13 ml for washings, 9 ml after elution with galactose) were eluted at a rate of 20 ml/h, and were pooled as indicated by the bars. Fractions with haemagglutinating activity are shaded. (*b*) Pooled fractions of peak III of (*a*) (72 ml, 1.35 g of protein) were applied to a column (27 cm × 1.4 cm) of acid-treated Sepharose 6B, previously equilibrated with phosphate-buffered saline. After washing, the column was eluted with 0.2 M-galactose in phosphate-buffered saline (arrow). Fractions (4 ml) were collected at a rate of 25 ml/h. Other details are as in (*a*).

ing powder was dried in the air. All subsequent operations were carried out at 2–4°C. The powder was extracted with 10 vol. of 0.2 M-NaCl containing 0.005 M-sodium phosphate buffer, pH 7.2. The suspension was left overnight with magnetic stirring, and was then centrifuged at 11000 *g* for 20 min. To this crude extract $(NH_4)_2SO_4$ was added slowly under constant stirring: the precipitates obtained at 30, 60 and 100% saturation of the salt were collected by centrifugation, dissolved in NaCl/sodium phosphate buffer and dialysed against the same solution. Most of the haemagglutinating and inhibitory activity was recovered in the 30–60%-satd.-$(NH_4)_2SO_4$ precipitate. From this, purification of the haemagglutinating lectin was attempted by affinity chromatography on a Sepharose 4B column, as described by Tomita *et al.* (1972). Part of the agglutinating activity was retained on the column, from which it could be eluted with 0.1 M-galactose (Fig. 1*a*). However, a considerable haemagglutinating activity was found in the effluent before elution with galactose: this activity could be absorbed completely on a column of acid-treated Sepharose 6B, from which it was eluted with 0.2 M-galactose (Fig. 1*b*). The purification of the lectin is summarized in Table 1.

The lectin purified in this way inhibited protein synthesis by a lysate of rabbit reticulocytes, as discussed below. However, the non-haemagglutinating material, which was not retained by Sepharose 6B, also showed a strong inhibitory activity in the lysate system. This material was concentrated by dialysis against solid sucrose, and then was dialysed overnight against 5 mM-sodium phosphate buffer, pH 6.5.

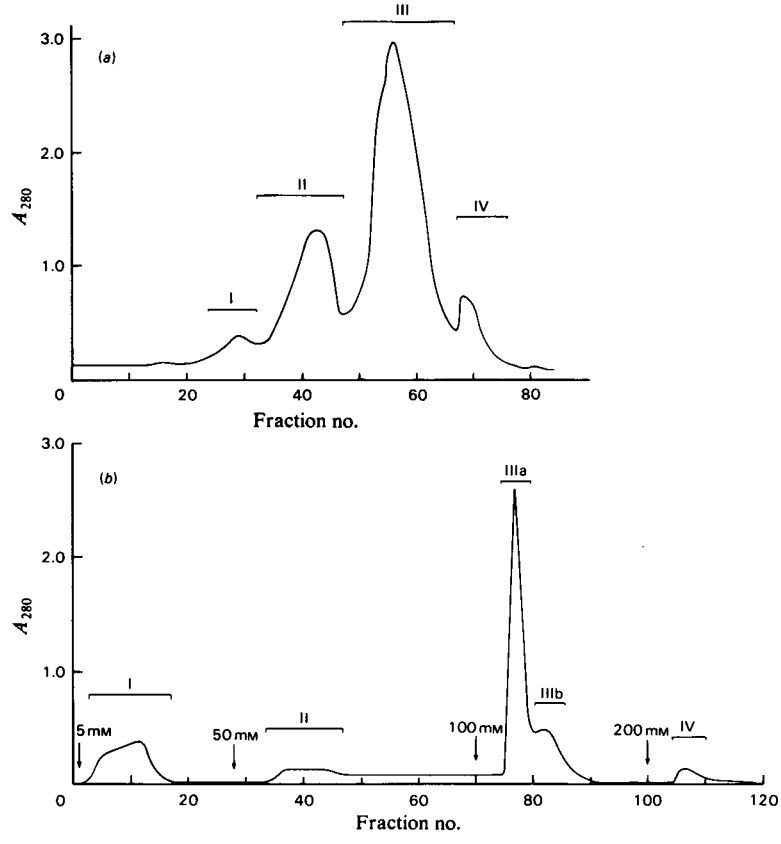

Fig. 2. *Purification of the Momordica charantia inhibitor*

(*a*) The material not retained by acid-treated Sepharose 6B (peak I of Fig. 1*b*) was concentrated as described in the text and dialysed against 5 mM-sodium phosphate buffer, pH 6.5. A portion (7.1 ml, containing 220 mg of protein) was applied to a column (84 cm × 1.5 cm) of Sephadex G-150 (superfine grade), previously equilibrated with the same solution, which was used also to elute the column. Fractions (2.7 ml) were eluted at a rate of 2.7 ml/h and were pooled as indicated by the bars. (*b*) Pooled fractions (23 ml, 84 mg of protein) of peak II of (*a*) were applied to a column (27 cm × 0.9 cm) of CM-Sephadex C-50, previously equilibrated with 5 mM-sodium phosphate buffer, pH 6.5. The column was eluted with increasing concentrations of sodium phosphate (arrows). Fractions (2.2 ml) were collected at a rate of 18 ml/h and were pooled as indicated by the bars.

Table 1. *Purification of Momordica charantia lectin and inhibitor*
Experimental conditions are described in the text.

		Haemagglutinating activity				Inhibitory activity on protein synthesis			
Preparation	Total protein (mg)	Specific activity (units/μg of protein)	Purification factor	$10^{-3}\times$Total activity (units)	Yield (%)	Specific activity ($ID_{50}/\mu g$ of protein)	Purification factor	$10^{-3}\times$Total activity (ID_{50} units)	Yield (%)
Crude extract	9794	0.29		2881	(100)	4.17		40808	(100)
$(NH_4)_2SO_4$ fractions									
0–30% satn.	704	0.13		90		1.47			
30–60% satn.	6235	0.51	1.75	3197	111	8.77	2.2	54694	134
60–100% satn.	69	Traces				1.48			
Sepharose 4B chromatography									
Unretained effluent:									
Peak I	15	Inactive				0.05			
Peak II	39	Inactive				0.09			
Peak III	885	Inactive				2.33			
Peak IV	4628	0.51	1.75	2373		25	6.2	115690	283
Eluate with 0.1 M-galactose	36	41	141	1475		0.58			
Sepharose 6B chromatography					241				
Unretained effluent	4154	Inactive				63	15	261702	641*
Eluate with 0.2 M-galactose	82	67	231	5467		0.32			
Sephadex G-150 chromatography									
Peak I	77					<10			
Peak II	310					515	129	158936	389
Peak III	401					<10			
Peak IV	4					<10			
CM-Sephadex C-50 chromatography									
Peak I	32					<10			
Peak II	33					<100			
Peak IIIa	86					562	140	48314	118
Peak IIIb	67					200		10331	25
Peak IV	18					<100			

* This step was repeated with similar results.

After further concentration in a Minicon B15 concentrator (Amicon Corp., Lexington, MA, U.S.A.) and further dialysis against 5 mM-sodium phosphate buffer, pH 6.5, the solution was applied on a Sephadex G-150 (superfine grade) column. From this column three peaks emerged, with the inhibitory activity almost entirely in the second peak (Fig. 2*a*). The active fractions were pooled, and were applied to a CM-Sephadex C-50 column, which was eluted stepwise with increasing concentrations of sodium phosphate buffer, pH 6.5. The inhibitory activity was eluted with 0.1 M-buffer as a sharp peak followed by a shoulder (Fig. 2*b*). The purification of the inhibitor is summarized in Table 1.

Electrophoresis, molecular weight and isoelectric point

Both the lectin and the inhibitor gave a single band on polyacrylamide-gel electrophoresis at pH 4.5, 7.0 and 9.5 (results not shown). Prior incubation at 37°C for 1 h in the presence of 1% 2-mercaptoethanol did not modify these electrophoretic patterns.

A single band was observed also when the freshly prepared lectin and inhibitor were subjected to electrophoresis in the presence of sodium dodecyl sulphate. Only with lectin preparations kept for some time at 2-4°C some minor bands appeared, corresponding to subunits and possibly to their aggregates (Fig. 3). The lectin preincubated with 2-mercaptoethanol showed four different subunits on gel electrophoresis in the presence of sodium dodecyl sulphate (Fig. 3), whereas the inhibitor was not modified.

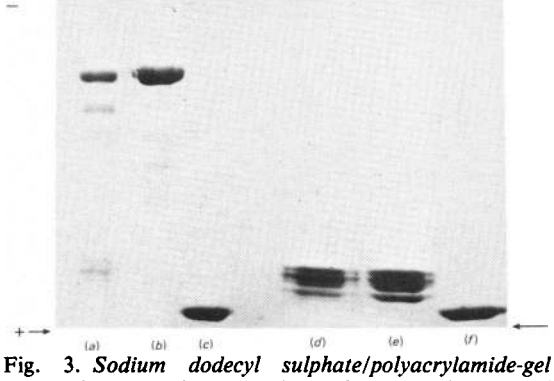

Fig. 3. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of Momordica charantia lectin and inhibitor*

Discontinuous electrophoresis on slab gels was performed as described in the Experimental section. (*a*) Lectin eluted from Sepharose 4B (25 μg); (*b*) lectin eluted from Sepharose 6B (25 μg); (*c*) inhibitor (25 μg); (*d*) lectin eluted from Sepharose 4B (25 μg) treated with 2-mercaptoethanol; (*e*) lectin eluted from Sepharose 6B (25 μg) treated with 2-mercaptoethanol; (*f*) inhibitor (25 μg) treated with 2-mercaptoethanol. Arrows indicate the Bromophenol Blue front.

The molecular weight of the lectin, as estimated by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, was 115000, and those of the lectin subunits were 30500, 29000, 28500 and 27000; that of the inhibitor was 23000.

The isoelectric points, as estimated by isoelectric focusing, were 6.0 for the lectin and 8.6 for the inhibitor.

Sugar content

The total neutral-sugar content of the lectin, expressed as galactose, was 116 μg/mg of protein (average of three determinations), and did not change after denaturation of the lectin with guanidinium chloride. No neutral sugar was detected in the inhibitor.

Toxicity

Both the lectin and the inhibitor were somewhat toxic to mice: the LD_{50} by the intraperitoneal route was 316 ± 1.4 μg/100 g body wt. (95% confidence limits 172–580) for the lectin, and 430 ± 1.3 μg/100 g body wt. (95% confidence limits 262–705) for the inhibitor. Deaths occurred between 12 and 72 h of poisoning, depending on the dose administered. No apparent effects were observed when the lectin was given intraperitoneally to rats at a dose of 1 mg/100 g body wt.

Haemagglutinating and mitogenic activity

The agglutinating activity of *Momordica charantia* lectin on erythrocytes from various species is shown in Table 2. Maximal activity was observed with human erythrocytes. In all cases except with rat erythrocytes the agglutinating activity was enhanced in the presence of neuraminidase.

Agglutination of human erythrocytes (group O)

Table 2. *Haemagglutinating activity of Momordica charantia lectin*

Experimental conditions are described in the Experimental section.

	Lowest concentration of lectin giving agglutination (μg/ml)	
Erythrocytes	Neuraminidase absent	Neuraminidase present
Human (group O, Rh^+)	0.024	0.006
Rabbit	0.049	Not tested
Rat	7.8	7.8
Pig	62.5	15.6
Calf	500	15.6
Horse	>500	1.95
Sheep	>500	31.2

was prevented by the following sugars, all tested at 50mM concentration, with the lectin at 100ng/ml: D-galactose, D-galactosamine, *N*-acetyl-D-galactosamine, D-fucose, 1-*O*-methyl α-D-glucopyranoside, 1-*O*-methyl β-D-glucopyranoside, lactose, melibiose and raffinose. The following sugars had no effect, under the same experimental conditions: D-arabinose, D-ribose, D-xylose, D-fructose, D-glucose, D-glucosamine, *N*-acetyl-D-glucosamine, cellobiose, maltose, sucrose, trehalose and melezitose.

The purified inhibitor at concentrations up to 250μg/ml did not agglutinate the erythrocytes of any of the species reported in Table 2, either in the presence or in the absence of neuraminidase.

Both the lectin and the inhibitor were not mitogenic to human peripheral blood lymphocytes cultured *in vitro*, at concentrations from 0.01 to 10μg/ml (lectin) and from 0.1ng to 50μg/ml (inhibitor).

Inhibition of protein synthesis

Cells. Momordica charantia lectin had a partial inhibitory effect on protein synthesis by Yoshida ascites cells at 100μg/ml, but not at 10μg/ml (Fig. 4). The inhibition was not modified in the presence of neuraminidase (25 units/ml; results not shown).

The inhibitor at concentrations up to 100μg/ml had no effect on protein synthesis by Yoshida cells (results not shown).

Reticulocyte lysate. Momordica charantia lectin was a strong inhibitor of protein synthesis by a rabbit reticulocyte lysate (Fig. 5). The ID_{50} (concentration giving 50% inhibition) of the freshly prepared lectin varied from one preparation to another, ranging from 0.6 to 45μg/ml, more usually from 1.5 to 15μg/ml (measured on ten preparations from the same seeds). On storage at 2–4°C the activity of the more-active preparations decreased, and that of the less-active ones increased, so that within a few days the ID_{50} of all preparations reached a stable value around 5μg/ml (average of ten preparations). Inhibition of protein synthesis by the less-active preparations was increased by preincubation at 37°C for 2h in the presence of 2-mercaptoethanol, whereas more-active and 'stabilized' preparations were not affected by the thiol. The inhibition was not affected by the presence in the reaction mixture of galactose at a concentration (0.1M) higher than that inhibiting agglutination. Once stabilized, the activity of the lectin was unchanged on storage at 2–4°C for several days, or at −25°C for a longer time, although in the

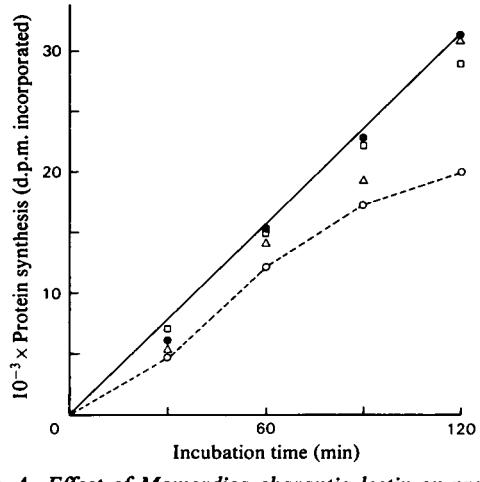

Fig. 4. *Effect of Momordica charantia lectin on protein synthesis by Yoshida ascites cells*
Yoshida AH-130 cells (4×10^6) were incubated at 37°C in 0.5ml of medium E 2a (Puck *et al.*, 1957) containing 5% calf serum and 0.5μCi of L-[^{14}C]-leucine, in the absence (●) or in the presence of *Momordica charantia* lectin, at a concentration of 1 (△), 10 (□) or 100 (○) μg/ml. Radioactivity incorporated was determined on 25μl samples.

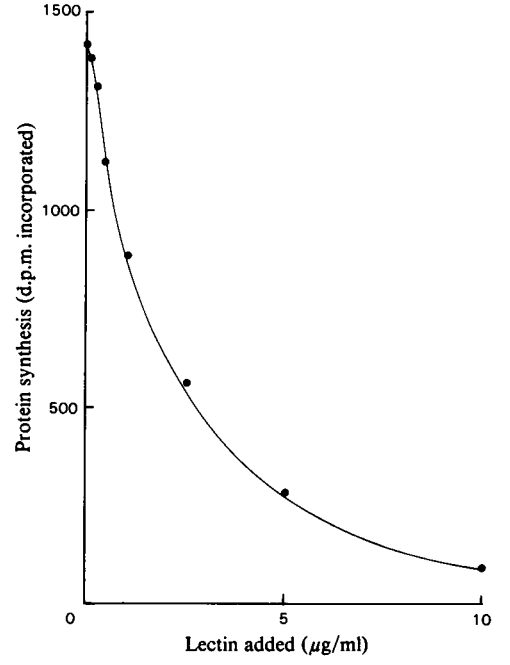

Fig. 5. *Effect of Momordica charantia lectin on protein synthesis by a rabbit reticulocyte lysate*
The reaction mixture contained, in a final volume of 0.25ml: 10mM-Tris/HCl buffer, pH7.4, 100mM-ammonium acetate, 2mM-magnesium acetate, 1mM-ATP, 0.2mM-GTP, 15mM-phosphocreatine, 12μg of creatine kinase, 0.05mM-amino acids (minus leucine), 0.75μCi of L-[^{14}C]leucine, the appropriate amount of lectin, and 0.1ml of lysate. Incubation was at 27°C for 5min. Radioactivity incorporated was determined on 25μl samples.

latter case it was considerably decreased after a few thawings, The lectin could be freeze-dried without loss of activity, although it became rather difficult to redissolve.

The purified inhibitor was over 1000-fold more active than the lectin in inhibiting protein synthesis by the lysate, the ID_{50} being 1.8 ng/ml (Fig. 6). This effect of the inhibitor was not affected by pretreatment with 2-mercaptoethanol. The activity of the inhibitor decreased slowly on storage at 2–4°C.

Ribosomes. *Artemia salina* ribosomes preincubated with the lectin or with the inhibitor and washed by centrifugation through 5% sucrose had a greatly decreased capacity to perform poly(U)-directed polymerization of phenylalanine (Table 3). Both proteins were effective at concentrations less than equimolar with ribosomes.

Protein synthesis by untreated ribosomes was unaffected on addition of ribosomes preincubated with the lectin, but was decreased when ribosomes pre-treated with the inhibitor were added. It cannot be excluded that this is due to traces of the inhibitor remaining attached to preincubated ribosomes.

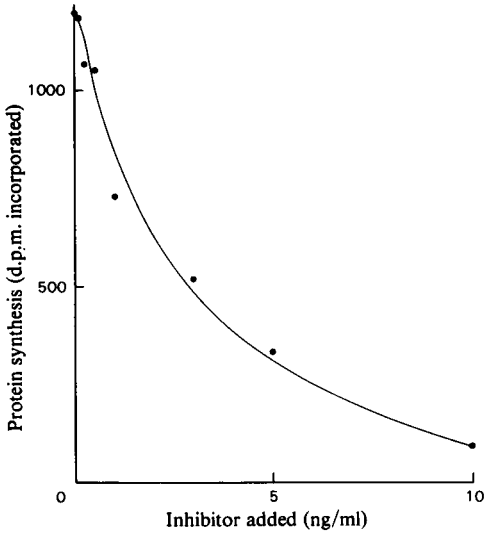

Fig. 6. *Effect of Momordica charantia inhibitor on protein synthesis by a rabbit reticulocyte lysate*
Experimental conditions were as described in the legend to Fig. 5.

Discussion

The seeds of *Momordica charantia* contain two proteins which are potent inhibitors of protein synthesis in cell-free systems, but have little or no effect on intact cells, thus accounting for the effect of seed extracts (Gasperi-Campani *et al.*, 1980).

One of these proteins is a haemagglutinating lectin inhibited by galactose, galactose-containing sugars and by α- and β-methylglucose; part is retained by Sepharose 4B (Tomita *et al.*, 1972) and the rest by acid-treated Sepharose 6B. The materials eluted from the two columns have identical properties, and thus it can be assumed they are a single protein, with higher affinity for Sepharose 6B than for Sepharose 4B. Hence the passage through Sepharose 4B can be eliminated from the purification procedure, as it was confirmed by the complete retention of the haemagglutinating activity on acid-treated Sepharose 6B obtained in subsequent preparations. The inhibitory activity of the freshly prepared lectin is rather variable, and is modified on storage at 0°C. This could be due to a rearrangement or to other modification(s) of the lectin molecule, such as the liberation of subunits and the formation of different aggregates, as suggested by the electrophoretic pattern (Fig. 3).

The second inhibitory protein (*Momordica charantia* inhibitor) has no haemagglutinating properties, and is one of the most potent inhibitors of

Table 3. *Effect of pretreatment of A. salina ribosomes with Momordica charantia lectin and inhibitor on poly(U)-directed [^{14}C]phenylalanine polymerization*
Artemia salina ribosomes (400 pmol) in 1 ml of 80 mM-Tris/HCl buffer, pH 7.4, containing 12 mM-KCl, 7 mM-magnesium acetate and 2 mM-dithiothreitol were incubated for 30 min at 24°C in the absence or in the presence of inhibitors at the indicated concentrations. After 3 h centrifugation at 105000 *g* through 1.5 ml of 5% (w/v) sucrose in the same buffer, the pellets were resuspended and poly(U)-directed ^{14}C phenylalanine polymerization was assayed on samples containing 3.75 pmol or 7.5 pmol of ribosomes.

Additions to preincubation mixtures	[^{14}C]Phenylalanine polymerized (pmol) by: Preincubated ribosomes (3.75 pmol)	Control ribosomes (3.75 pmol) plus preincubated ribosomes (3.75 pmol)
None	7.44	8.74
Lectin (450 pmol)	0.72	8.31
Lectin (75 pmol)	2.35	8.76
Inhibitor (350 pmol)	0.29	3.21
Inhibitor (70 pmol)	0.25	5.77

protein synthesis hitherto described, more potent than the A chains of ricin, abrin and modeccin, and second only to the *Phytolacca americana* peptide (Irvin, 1975) which in our lysate system had $ID_{50} < 1$ ng/ml.

The recovery of the lectin and especially of the inhibitor was higher than 100% (see Table 1). A similar phenomenon was observed by Ersson *et al.* (1973) and by Ersson (1977) during the purification of the lectin from *Crotalaria juncea*, and was attributed to removal of interfering substances present in crude extracts. The possibility must also be considered that the proteins could be modified and rendered more active by the purification procedure.

Both proteins act by altering ribosomes, and making them unable to perform protein synthesis. As with ricin (Montanaro *et al.*, 1973) and modeccin (Montanaro *et al.*, 1978), no cofactors are necessary for the inactivation of ribosomes. This effect of the proteins seems to be catalytic, i.e. enzymic, since they inactivate more than one molar equivalent of ribosomes. Thus these proteins seem to act in a similar way to ricin and related toxins, from which, however, they differ in being much less toxic to animals or to whole cells. The toxicity of ricin and of the other toxic lectins results from the combined action of both their constituent A and B subunits: the B subunit binds to cells allowing the penetration of the A subunit, which acts on ribosomes (Olsnes & Pihl, 1977). *Momordica charantia* lectin consists of four different subunits, of which at least one should have the A function and inhibits protein synthesis, whereas at least one should behave like a B subunit capable of binding to cells, as demonstrated by the haemagglutinating activity. It is likely that the low toxicity of *Momordica charantia* lectin is because the properties of its B subunit differ from those of the B chain of other more toxic lectins, or because of a peculiar binding of the B and other subunits with the A chain, which does not allow the latter to enter cells. Considerations of the same kind lead us to suppose that the inhibitor should be similar to an A chain, and this would account (i) for the lack of effect on whole cells, and consequently for the low toxicity to animals, and (ii) for the lack of haemagglutinating activity. It should be considered also that the inhibitor cannot be a subunit of the lectin, since its molecular weight is lower than that of any subunit. It is possible, however, that it is a precursor or a derivative of the active subunit of the lectin.

Lin *et al.* (1978) isolated from the seeds of *Momordica charantia* two lectins, called *Momordica charantia* agglutinin and momordin. Both agglutinated erythrocytes and momordin inhibited protein synthesis by Ehrlich ascites cells, at relatively high concentrations. The molecular weights of these lectins are 32000 (agglutinin) and 24000 (momordin), and it is possible that they are subunits of the lectin that we isolated, separated during the purification procedure. Momordin has practically the same molecular weight as the inhibitor; the latter, however, has no haemagglutinating activity and does not inhibit protein synthesis by intact cells.

The property of inhibiting protein synthesis is common to several lectins and proteins from plants (see the introduction). For the inhibitory proteins purified from *Momordica charantia* seeds, as in all other cases studied so far, the inhibitory activity is due to ribosomal damage, which seems to be brought about enzymically.

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